

**TOXIN GENES FROM THE BACTERIA XENORHABDUS NEMATOPHILUS
AND PHOTORHABDUS LUMINESCENS**

Field of the Invention:

5 The present invention concerns the identification and isolation of a
new class of protein toxins with specificity for insects, which are produced
by bacteria from the genera *Xenorhabdus* and *Photorhabdus*. In addition, the
present invention relates to the incorporation of genes encoding this class of
10 toxin into, for example, insect-specific viruses (including entomopox and
nuclear polyhedrosis viruses), bacteria (including *Gracilicutes*, *Firmicutes*,
Tenericutes and *Mendosicutes*), yeast and plants for control of insect pests.

Background of the Invention:

15 Insect pathogenic nematodes of the families *Steinernematidae* and
Heterorhabditidae are known to be symbiotically associated with bacteria of
the genera *Xenorhabdus* and *Photorhabdus* respectively. It has been observed
that these bacteria have the ability to kill a wide range of different insects
without the aid of their nematode partners. The present inventors have
isolated polynucleotide molecules encoding a new class of protein
20 insecticidal toxins from *Xenorhabdus nematophilus* strain A24 and
Photorhabdus luminescens strain V16/1.

Disclosure of the Invention:

25 In a first aspect, the present invention provides an isolated
polynucleotide molecule encoding an insecticidal toxin, said polynucleotide
molecule comprising a nucleotide sequence which substantially corresponds
to the nucleotide sequence shown as SEQ ID NO: 1 or SEQ ID NO: 2.

30 In a second aspect, the present invention provides an isolated
polynucleotide molecule encoding an insecticidal toxin, said polynucleotide
molecule comprising a nucleotide sequence having at least 85%, more
preferably at least 95%, sequence identity to the nucleotide sequence shown
as SEQ ID NO: 2.

35 In a third aspect, the present invention provides an insecticidal toxin,
in a substantially pure form, which toxin comprises an amino acid sequence
having at least 95% sequence identity to that shown as SEQ ID NO: 3.

In a fourth aspect, the present invention provides an insecticidal toxin, in a substantially pure form, which toxin comprises an amino acid sequence having at least 85%, more preferably at least 95%, sequence identity to that shown as SEQ ID NO: 4.

5 Most preferably, the insecticidal toxin of the third or fourth aspect comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 3 or SEQ ID NO: 4 respectively.

10 In a fifth aspect the present invention provides a recombinant microorganism, the recombinant microorganism being characterised in that it is transformed with and expresses the polynucleotide molecule of the first or second aspects of the present invention.

15 The microorganisms which may be usefully transformed with the polynucleotide molecule of the first or second aspects of the present invention include bacteria, such as *Escherichia*, *Gracilicutes*, *Firmicutes*, *Tenericutes* and *Mendosicutes*; protozoa and yeast. The microorganism can be transformed by routine methods using expression vectors comprising the toxin-encoding polynucleotide molecule operably linked to a suitable inducible or constitutive promoter sequence.

20 In a sixth aspect, the present invention provides a method of producing an insecticidal toxin, said method comprising:

(i) culturing a microorganism according to the fourth aspect under conditions suitable for the expression of the toxin-encoding polynucleotide molecule, and

(ii) optionally recovering the expressed insecticidal toxin.

25 In a seventh aspect, the present invention provides a recombinant insect-specific virus, the recombinant insect-specific virus being characterised in that it includes within a non-essential region of its genome the polynucleotide molecule of the first or second aspects of the present invention operably linked to a suitable inducible or constitutive promoter sequence.

30 The recombinant insect-specific virus of the seventh aspect is preferably selected from entomopox and nuclear polyhedrosis viruses. The recombinant virus can be produced by routine methods such as homologous recombination.

35 In an eighth aspect, the present invention provides a method for killing pest insects, said method comprising applying to an area infested with said

insects an effective amount of a recombinant microorganism according to the fourth aspect and/or a recombinant virus according to the seventh aspect, optionally in admixture with an acceptable agricultural carrier.

In a ninth aspect, the present invention provides a plant transformed with, and capable of expressing, the polynucleotide molecule of the first or second aspects of the present invention.

The plant according to the ninth aspect may be any plant of agricultural, arboricultural, horticultural or ornamental value that is susceptible to damage by feeding pest insects. However, preferably, the plant is selected from plants of agricultural value such as cereals (e.g.; wheat and barley), vegetable plants (e.g.; tomato and potato) and fruit trees (e.g., citrus trees and apples). Other preferred plants include tobacco and cotton.

The plant can be transformed by routine methods including *Agrobacterium* transformation and electroporation. Preferably, the toxin-encoding polynucleotide molecule is operably linked to a suitable inducible or constitutive promoter sequence. Particularly preferred promoter sequences include the cauliflower mosaic virus (CaMV 35 S) promoter element and promoter elements from the sub-clover stunt virus (SCSV).

The term "substantially corresponds" as used herein in relation to the nucleotide sequence is intended to encompass minor variations in the nucleotide sequence which due to degeneracy do not result in a change in the encoded protein. Further this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded protein.

The term "substantially corresponding" as used herein in relation to the amino acid sequence is intended to encompass minor variations in the amino acid sequence which do not result in a decrease in biological activity of the insecticidal toxin. These variations may include conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and P, α -alkalamino acids.

The term "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature or group of steps, components or features with or

without the inclusion of a further step, component or feature or group of steps, components or features.

The invention will hereinafter be further described by way of reference to the following, non-limiting example and accompanying figures.

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Brief description of the accompanying figures:

Figure 1: Nucleotide sequence of the protein coding (sense) strand of the *X. nematophilus* DNA insert of clone toxb4. The translation initiation codon (ATG) at nucleotide position 17-19 and the translation termination codon (TAA) at nucleotide position 1121-1123 are indicated by shaded boxes.
Locations of oligonucleotide sequences used for sequencing primer design are indicated by arrows and a primer name (TOX F1, TOX R3 etc.). Arrows directed left-to-right, positioned above the sequence indicate sense-strand primers, arrows directed right-to-left, positioned below the sequence indicate anti-sense primers.

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Figure 2: Deduced sequence of the 368 amino acid toxb4 protein from *X. nematophilus* strain A24, derived by conceptual translation of the long open reading frame commencing at nucleotide position 17 and ending at nucleotide position 1120 of the *toxb4* gene sequence (Fig. 1).

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Figure 3: Restriction map of *P. luminescens* V16/1 toxin gene clone showing location of putative toxin protein coding region (solid black box) and direction of transcription (arrow). RI=*EcoRI*, RV=*EcoRV*, H=*Hind III*, S=*Sma I*. Toxin production from clones containing selected restriction fragments is indicated above the restriction map (+, toxin activity; -, no toxin activity).

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